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MEETING REVIEW

FDA Public Workshop Summary: Advancing Animal Models for Antibacterial Drug Development

James M. Byrne,[a,b] Ursula Waack,[a,b] Edward A. Weinstein,[a] Abhay Joshi,[c] Simone M. Shurland,[a] Dmitri Iarikov,[a] Jürgen B. Bulitta,[d] Binh An Diep,[e] Tina Guina,[f] William W. Hope,[g] Matthew B. Lawrenz,[h] Alexander J. Lepak,[i] Brian M. Luna,[j] Lynn Miesel,[k] Andrew J. Phipps,[l] Thomas J. Walsh,[m] William Weiss,[n] Thushi Amini,[a] John J. Farley[a]

[a]Office of Infectious Diseases, Office of New Drugs, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, Maryland, USA
[b]Oak Ridge Institute of Science and Education, Oak Ridge, Tennessee, USA
[c]Office of Clinical Pharmacology, Office of Translational Sciences, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, Maryland, USA
[d]Department of Pharmacotherapy and Translational Research, College of Pharmacy, University of Florida, Orlando, Florida, USA
[e]Division of HIV, Infectious Diseases and Global Medicine, Department of Medicine, University of California, San Francisco, San Francisco, California, USA
[f]Biomedical Advanced Research and Development Authority, Office of the Assistant Secretary for Preparedness and Response, Department of Health and Human Services, Washington, DC, USA
[g]Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, United Kingdom
[h]Center for Predictive Medicine for Biodefense and Emerging Infectious Diseases, Department of Microbiology and Immunology, University of Louisville School of Medicine, Louisville, Kentucky, USA
[i]Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA
[j]Department of Molecular Microbiology and Immunology, Keck School of Medicine of USC, Los Angeles, California, USA
[k]Pharmacology Discovery Services, Ltd., New Taipei City, Taiwan
[l]Tunnell Government Services, Biomedical Advanced Research and Development Authority, Washington, DC, USA
[m]Transplantation-Oncology Infectious Diseases Program, Departments of Medicine, Pediatrics, and Microbiology & Immunology, Weill Cornell Medicine of Cornell University, New York, New York, USA
[n]PreClinical Services, UNT System College of Pharmacy, Fort Worth, Texas, USA

ABSTRACT The U.S. Food and Drug Administration (FDA) hosted a public workshop entitled "Advancing Animal Models for Antibacterial Drug Development" on 5 March 2020. The workshop mainly focused on models of pneumonia caused by *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The program included discussions from academic investigators, industry, and U.S. government scientists. The potential use of mouse, rabbit, and pig models for antibacterial drug development was presented and discussed.

KEYWORDS FDA, animal models, antibacterial, antibiotics, drug development, humanized dose, pneumonia, regulatory, workshop

There is an urgent need for new antibacterial drugs active against pathogens associated with drug resistance and poor clinical outcomes. *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are examples of key pathogens identified by the Centers for Disease Control and Prevention (CDC) in their Antibiotic Resistance Threats Report (1). These pathogens have been challenging to study in clinical trials. Strategies to streamline development of antibacterial drugs that target single species were discussed in a U.S. Food and Drug Administration (FDA) public workshop in 2016 (https://www.fda.gov/drugs/news-events-human-drugs/facilitating-antibacterial-drug-development-patients-unmet-need-and-developing-antibacterial-drugs), and the potential for animal models to provide supportive data was subsequently discussed in a 2017 workshop (https://www.fda.gov/drugs/news-events-human-drugs/current-state-and-further-development-animal-models-serious-infections-caused-acinetobacter). The 2017 workshop highlighted limitations of existing small-animal models for certain bacterial

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Address correspondence to James M. Byrne, James.Byrne@fda.hhs.gov.

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infections such as ventilator-associated pneumonia. Such limitations include the difficulty of reproducing aspiration models of infection, feasibility of long-term mechanical ventilation, limited ability to provide critical care, and the financial burden of developing labor-intensive models. Several research contracts were awarded for the development of animal models of bacterial pneumonia caused by *P. aeruginosa* and *A. baumannii* (https://www.fda.gov/media/139452/download). The progress on these animal models was presented at a follow-up workshop, held on 5 March 2020, at the FDA White Oak campus in Silver Spring, MD (https://www.fda.gov/drugs/news-events-human-drugs/advancing-animal-models-antibacterial-drug-development-03052020-03052020). This summary presents a high-level overview of the themes and topics discussed during the workshop with an emphasis on the commentary from the panel discussion. The workshop included panel members from industry, academia, and the federal government.

SCIENTIFIC LANDSCAPE, CLINICAL PHARMACOLOGY, AND DEVELOPMENT RESOURCES

Dr. Ursula Waack (FDA) described her findings from a systematic review of animal models of bacterial pneumonia used in regulatory submissions for investigational new drugs (INDs) to the Division of Anti-Infectives from 2000 to 2019 (2). Neutropenic murine models were the most common models utilized in studies of drug activity. Mice were commonly selected for experiments with shorter durations, such as 24 h. Experiments lasting longer than 24 h in duration were more likely to use other animal species, such as rat or rabbit. None of the submissions utilized a ventilator-associated model of pneumonia. Dr. Waack's analysis found that there is potential for harmonization in animal model study design components, such as in the bacterial species and strains used, the time to start treatment, and the endpoints utilized. The use of standardized models would reduce variability across studies and allow for comparative assessments of antimicrobial drugs. The analysis of peer-reviewed published studies of animal models of bacterial pneumonia showed that a wider variety of models were used, including pigs and guinea pigs, in those studies than in studies included in regulatory submissions. The published studies showed trends similar to the data from models submitted to the FDA in terms of predominant animal model used, length of time of studies, and study endpoints. One notable difference was that only a few published animal model studies were able to incorporate mechanical ventilation into the design. This difference represents a gap in drug development where drug developers could develop or refine a model of ventilator-associated pneumonia to study the disease in its most severe form and relevant to the human disease manifestation. Additionally, the published studies used immunocompetent models whereas most of the IND-submitted studies used neutropenic models.

Dr. Abhay Joshi (FDA) provided an overview of pharmacokinetic (PK) considerations for selecting dosing regimens for animal infection model experiments that are conducted during the late stages of drug development. The term "late stage" was defined as the point in a drug development program when a clinical dosage regimen for clinical efficacy studies has been determined. Dr. Joshi described two approaches for selecting a dosing regimen for these animal models. One approach is based on matching the extent of bacterial killing desirable in clinical settings, and the other approach is based on adjusting the free drug exposure in the animal model to a level similar to that anticipated in humans who receive a clinical dosage regimen (often referred to as "humanized dosing"). Selecting a dosing regimen based on humanized dosing was noted to be advantageous as this approach (i) avoids uncertainties associated with the determination of PK-pharmacodynamic (PD) target value estimates, which are relied upon to infer that bacterial killing is matched, and (ii) mimics the overall free drug exposure cycles anticipated in humans and thereby covers scenarios when the drug exposure-bacterial killing relationship is unknown or is best described by a mechanistic relationship other than the routinely used PK-PD indices (e.g., the area under the free concentration-time curve [*f*AUC]/MIC ratio, the maximum free concentration of drug in

serum [fC_{max}]/MIC, percent time above the MIC [$fT > MIC$]). Based on those two advantages, the humanized dosing approach is considered a conservative approach. Subsequently, two dosing strategies (i.e., continuous intravenous [i.v.] infusion and intermittent dosing) to administer humanized dosing in animal models were presented and discussed along with the potential advantages and challenges represented by each strategy. Dr. Joshi also provided an example for each dosing strategy using preliminary meropenem PK data from ongoing rabbit and murine model research. In the rabbit model example, human-equivalent meropenem exposures associated with a 3-h infusion were attempted using programmable infusion pumps, delivering five different doses for different time intervals over a total of 8 h, which represented a single clinical dosing interval. In the murine model example, an attempt was made to match human-equivalent meropenem exposure over 8 h using intermittent dosing comprised of three different bolus doses (descending dose levels). Dr. Joshi noted that additional research is being considered to further optimize meropenem dosing in the murine model to match human-equivalent exposures. As a final point, Dr. Joshi emphasized the importance and critical role of bioanalytical method validation, serum protein binding assessments, and dose-ranging PK experiments.

Dr. Judy Hewitt (National Institutes of Health [NIH]/National Institute of Allergy and Infectious Diseases [NIAID]) described drug development support tools and services offered by NIAID to assist in various stages of drug development. NIAID provides technical assistance as well as testing services for candidate therapeutics through their portfolio of preclinical infectious disease models, ranging from mice to nonhuman primates (https://www.niaid.nih.gov/research/pre-clinical-models-infectious-disease). Dr. Hewitt noted that the NIAID's BEI Resources Repository can supply organisms and reagents to the microbiology and infectious diseases research community (https://www.beiresources.org/). Dr. Tina Guina (Department of Health and Human Services [HHS]/Biomedical Advanced Research and Development Authority [BARDA]) presented opportunities for collaboration and funding of innovative antibacterial drug development programs provided through a global public-private partnership, Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerators (CARB-X) (https://carb-x.org/), and the services BARDA offers through their Nonclinical Development Network.

MURINE MODELS OF PNEUMONIA

Dr. Lynn Miesel (Pharmacology Discovery Services) presented ongoing work supported by the NIH to develop a neutropenic murine *P. aeruginosa* model utilizing two *P. aeruginosa* strains from the Centers for Disease Control and Prevention (CDC) and FDA Antibiotic Resistance (AR) Isolate Bank (https://wwwn.cdc.gov/arisolatebank/). These strains were selected due to their virulence properties and different resistance profiles against carbapenems. For both strains, natural history studies were presented describing the effect of inoculum size and route of infection. Next, the effect of time to intervention was explored with amikacin and colistin as reference treatments. Animals were observed over a period of 120 h, and a change in body temperature was presented as a potential, compassionate endpoint that may reliably predict mortality. Ongoing work will expand the murine model to additional pathogens and will explore an immunocompetent model.

A presentation by Dr. Brian Luna (University of Southern California) focused on refining an oral aspiration pneumonia murine model of *A. baumannii* infection (3) as well as a murine bloodstream infection model. Dr. Luna determined an infectious dose and conducted a natural history study (4) with three different *A. baumannii* strains. These strains have different profiles of susceptibility to the three test antibiotics: amikacin, meropenem, and polymyxin B. For the presented efficacy studies using amikacin, the trigger to treat was 2 h postinfection. The presence of disease at this time point was confirmed with histopathological analysis of the lungs in the natural history studies. In addition to pathology, various biomarkers were also assessed during the natural history study. Future work will focus on assessing the efficacy of the three antibiotics in both models. Dr. Luna's collaborator, Dr. Jürgen Bulitta (University of

Florida), described the population PK modeling methods and approaches for determining humanized dosing regimens for amikacin, polymyxin B, and meropenem. Dr. Bulitta emphasized the need for evaluating PK in infected animals using the same isolates and pathogens that would be used in animal efficacy studies. Dr. Bulitta also discussed the challenges encountered in determining the humanized dosing regimen for meropenem in the murine model and explained that it is very labor intensive to attain human-equivalent meropenem exposure. The same issue applies to other beta-lactams with a short half-life in mice. Future plans include testing to determine whether the addition of probenecid or cilastatin would increase the half-life of meropenem in mice, which may allow for more closely matching and attaining human-equivalent meropenem exposure.

Dr. Matthew Lawrenz (University of Louisville) built upon previously published studies of murine *P. aeruginosa* pneumonia (5) with a discussion of 50% lethal dose (LD_{50}) data for four strains in the CDC and FDA AR Isolate Bank. An infectious dose of $10\times$ LD_{50} was selected as a challenge dose in the neutropenic model, with a trigger-to-treat time point of 3 h postinfection and an endpoint of survival at 21 h. Lung histopathological analysis showed that at the trigger-to-treat time point, there were no observable changes. Dr. Lawrenz's future plans include investigating additional time points to select a trigger-to-treat time supported by pathology. It was noted that additional information on the clinical context of isolates provided by the CDC and FDA AR Isolate Bank, such as the body site where the isolate was obtained and type of infection, could assist researchers in selecting strains that may more closely mimic the clinical environment.

Dr. Alex Lepak (University of Wisconsin) discussed the aztreonam PK experiments and challenges in determining the appropriate drug dosing for mice in Dr. Lawrenz's model. Dr. Lepak explained how aztreonam dosing can be determined by prioritizing the PK-PD driver rather than mimicking the human drug exposure curve by a complex dosing regimen, which may bring its own technical and feasibility challenges due to the very short elimination half-life of aztreonam in mice.

NONMURINE ANIMAL MODEL DEVELOPMENT

Larger-animal models of infection, including meningoencephalitis, nonventilated pneumonia, and ventilator-associated pneumonia (VAP), were discussed. Dr. William Hope (University of Liverpool) discussed a rabbit model of meningoencephalitis caused by *P. aeruginosa*. This 30-h model consisted of intrathecally inoculating rabbits with *P. aeruginosa* followed by treatment with intravenous meropenem or tobramycin 6 h postinoculation. The endpoint was bacterial burden in both central nervous system (CNS) subcompartments, e.g., cerebrum and cerebrospinal fluid (CSF). A population PK-PD modeling approach was utilized to link both drugs' exposure in plasma and CSF to bacterial killing in CSF. For meropenem, Dr. Hope reported that a dose-exposure response could be observed and that drug exposures in plasma and CSF (measured as the area under the concentration-time curve) were found to be associated with bacterial killing. A similar association between dose/exposure and bacterial killing was not observed for tobramycin.

Dr. Hope noted that CNS involvement is often assumed in neonatal infections and that the collection of data to determine an appropriate dosing for neonatal patients is challenging. This model aimed to define the PD of antibacterial agents in the rabbit brain and in related subcompartments. Dr. Hope emphasized that demonstration of a drug's presence in CSF does not necessarily correlate with meaningful clinical activity. Additional discussion focused on the potential for this model to inform regarding a drug's activity in CNS compartments and, thereby, aiding the development of new antibiotics for neonates by providing supportive evidence for dosage justification.

Dr. Thomas Walsh (Weill Cornell Medicine of Cornell University) presented two persistently neutropenic rabbit models of Gram-negative pneumonia. The models examined the pathogenesis, host response, and treatment of pneumonia caused by susceptible and resistant strains of *P. aeruginosa* and *Klebsiella pneumoniae* (6, 7). The

animals were inoculated endotracheally to allow for bacterial colonization throughout the tracheobronchial mucosa. The model can study a treatment course of up to 14 days' duration, and central venous access allows for serial blood sampling. Endpoints include measurement of bacterial burden in lung tissue and the alveolar space by bronchoalveolar lavage, lung weights as a marker for organism-mediated pulmonary injury, and inflammatory biomarkers. The model requires supportive care being provided throughout the immune impairment, similarly to that provided to immunocompromised patients. Pneumonia caused by *P. aeruginosa* was treated with a humanized dosage of ceftolozane-tazobactam resulting in a $\geq 10^5$ CFU reduction in residual pulmonary and bronchoalveolar lavage (BAL) fluid bacterial burdens. This antibacterial activity coincided with a reduction of lung weight. Survival also was prolonged in the ceftolozane-tazobactam treatment groups in comparison to an untreated control group.

Dr. Binh Diep (University of California, San Francisco) presented models of acute nonventilated pneumonia and VAP caused by *P. aeruginosa* by the use of immunocompetent rabbits. Natural history studies were conducted for both models to determine whether they mimic human disease and to identify the optimal time to initiate treatment. Dr. Diep noted that the VAP natural history study showed that infected rabbits developed pathophysiology of acute respiratory distress syndrome (ARDS), including progressive decreases in the PaO_2/FiO_2 ratio (the ratio of arterial oxygen partial pressure [PaO_2 {mm Hg}] to fractional inspired oxygen), bilateral neutrophilic infiltrate, and severe hypotension, whereas uninfected control rabbits ventilated with low tidal volume developed only minimal ventilator-induced lung injury. Survival was the primary endpoint, and bacterial burden and the lung weight-to-body weight (LW/BW) ratio were secondary endpoints in both models. The VAP model also used biomarkers, such as the PaO_2/FiO_2 ratio and lactate values, as secondary endpoints. A humanized meropenem regimen was used for model validation. The nonventilated model compared meropenem with placebo. The VAP model included meropenem alone, meropenem plus fluid challenge (FC) plus norepinephrine (NE), FC plus NE, and normal saline arms. The VAP model required extensive physiological monitoring and an experimental intensive care unit (ICU) setup.

Dr. Diep stated that these rabbit models mimic human nonventilated and ventilated bacterial pneumonia. He discussed the results showing that meropenem increased survival and improved secondary endpoints in both models. In the VAP model, a significantly greater number of animals in both meropenem arms survived the entire duration of the 36-h experiment. None of the animals in the control arms survived the length of the experiment. Bill Weiss (University of North Texas) discussed the humanized dosing regimen for meropenem for acute pneumonia using intermittent short infusions and the VAP rabbit models using staggered continuous infusions.

The last presentation was by Dr. Andrew Phipps, a contractor for BARDA. He described pilot studies of a porcine ventilator-associated pneumonia model of *A. baumannii* or *P. aeruginosa*. Each study confirmed that mechanical ventilation in large animals such as pigs is possible up to 96 h. Natural history studies are needed at the current stage of development of this model.

PANEL DISCUSSION

The endpoints used in model development were discussed by the panelists. It was noted that selection of the endpoint would depend on the objectives of the studies. Clinical endpoints, such as mortality and signs of disease reflective of symptoms that are experienced by patients, make the model more relevant to the human condition and readily translate to supportive information for clinical trials. Microbiological endpoints, such as bacterial burden, are useful to identify and characterize a relationship between a drug's exposure and microbiological clearance. It was also noted that solely reducing bacterial burden in a model may not track closely with resolution of clinical symptoms. Panelists indicated that disease may manifest differently in animals than in humans and that it may be challenging to recapitulate human disease.

Panelists agreed that the natural history of a model should be understood before

assessing the treatment effect of antibacterial agents. Natural history studies are important when establishing a trigger-to-treat time point that is consistent with treatment of disease rather than prophylaxis of infection or when a disease has barely established. When discussing criteria used for determining the trigger-to-treat, a number of options were presented. One of the options is to identify disease via histopathology and link the histopathologic findings to clinical symptoms and to the time after bacterial inoculation. This may also include the measurement of other disease markers, such as blood gas analysis. The use of clinical criteria, such as fever, hypothermia, or other relevant biomarkers, as a trigger to treat was considered appropriate when evaluating the effectiveness of an antibacterial agent. The use of bacterial burden was considered an important endpoint in both efficacy studies and PK-PD studies.

Panelists discussed the complementary nature of the models. If a drug developer understands and optimizes an investigational agent in a murine model, the development process could be furthered by evaluating the drug in another model. Assessing a drug effect in multiple models may build confidence in the experimental results. It was noted that creating very specific models may hinder potential drug development opportunities by screening out novel antibacterial agents early in development, especially for nontraditional antibacterial agents. The panel highlighted the need for guidelines to describe areas of scientific consensus, help investigators avoid common pitfalls, and help determine the type of animal model to use when profiling novel antibacterial agents.

Finally, panelists discussed the identification and characterization of bacterial species to be used in an animal model. Pathogen virulence in humans is not the same as the virulence seen in animal models. Often, there is a reliance on nonpathogenic laboratory strains which may not be clinically relevant. Sources for reference pathogen strains included the NIAID's BEI Resource Repository and the CDC and FDA AR Isolate Bank. Multiple panelists stated that having access to a defined, curated set of human clinical isolates would be helpful for attempting to replicate the human disease state in a model. Currently, the CDC and FDA AR Isolate Bank does not provide clinical information about the source of infection when researchers are selecting strains. The ability to select a strain by infection source would be valuable to the animal model development community.

POTENTIAL REGULATORY IMPACTS

The primary data for regulatory decision-making are based upon adequate and well-controlled clinical trials. Animal studies have the potential to supplement the clinical data in useful and meaningful ways. Two recently approved drugs, pretomanid and cefiderocol, are examples. In the case of pretomanid, a clinical trial was conducted to demonstrate the effectiveness of a regimen consisting of bedaquiline, linezolid, and pretomanid to treat extensively drug-resistant, treatment-intolerant, or nonresponsive multidrug-resistant pulmonary tuberculosis. It was unfeasible and unacceptable to perform a factorial clinical trial design to assess the contribution of each drug in the regimen. Therefore, animal studies were used to assess the contribution of individual components of the regimen. In the case of cefiderocol, indicated for the treatment of urinary tract infections in adults with limited or no alternative treatment options, animal studies were included in the prescribing information (https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/209445s000lbl.pdf) describing drug activity against important pathogens that are an infrequent cause of infection, such as *Stenotrophomonas maltophilia* and *Burkholderia cepacia*. In addition, animal studies provided supporting data for drug activity versus certain bacterial resistance phenotypes (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/209445Orig1s000MultidisciplineR.pdf).

In the setting of antibacterial drug development to address unmet need due to antimicrobial resistance, the data from animal studies are not intended to replace clinical trial data. Instead, supportive animal studies are expected to accompany adequate and well-controlled clinical trials, with the animal data providing some

information regarding the activity of the drug in situations where obtaining clinical data would be highly impractical. Factors to be considered in designing such supportive animal studies include the selection of bacterial strains based on clinical relevance (i.e., rare bacterial species associated with the indication or with particular antibacterial drug resistance phenotypes), utilizing a humanized dosing strategy to evaluate a drug's activity with human-equivalent exposures, and selecting the endpoints that are clinically relevant and scientifically sound.

CONCLUSION

Animal models of bacterial infection provide useful information at various points during a drug development program. Early in development, a common use of these models is to provide proof-of-concept *in vivo* activity data. Exposure-response studies contribute to the understanding of PK and PD parameters of antibacterial drugs. When combined with data from toxicology studies, animal models provide information on potential toxicities in humans and inform dosing in clinical trials. While every effort should be made to perform human clinical trials across the spectrum of human disease, animal models of serious bacterial infection are useful to explore the activity of a candidate antibacterial drug targeting a single species or less commonly occurring bacterial pathogens and may be further developed to help to predict activity in humans.

The workshop highlighted advancements in animal model research such as the importance of conducting natural history studies early on in animal model development. Studies can more closely mimic clinical trials by using humanized dosing and endpoints which are aligned with those used in clinical trials. The research presentations and the discussions during the workshop also highlighted potential limitations of using animal models. The data from these studies can be highly variable and questions remain about the reproducibility of the data. Elements of animal models in general could be harmonized to help improve reproducibility and support future study design decisions. By standardizing and refining animal models, drug developers can increase the amount of data gathered from a smaller number of animals. Additionally, using a standardized model will allow for comparison between drug programs, reducing the need for replicating animal studies.

Future work in this field should further optimize the use of animal models at different stages of the drug development process as well as investigate various predictive aspects of the animal study design such as the different dosing strategies. Additionally, animal models presented at the workshop focused on FDA-approved antibiotics that are well characterized, whereas antibiotics that operate by new mechanisms of action may require development of additional animal models.

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